A MICRURGICAL STUDY OF CROWN GALL INFECTION IN TOMATO[1]

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INTRODUCTION

This paper is concerned with micrurgical studies on crown gall infection in tomato (*Lycopersicon esculentum* Mill.), with emphasis on the relation of inoculation technique to infection. Despite a voluminous literature on crown gall (*11*),[2] little is known of the quantitative aspect of the infection mechanism, particularly the limits in wound size and in bacterial numbers that govern infection.

LITERATURE REVIEW

Levine (*9*) studied the effect of inoculating tobacco plants with various quantities of different dilutions of the crown gall organism (*Phytomonas tumefaciens* (Sm. and Town.) Bergey et al.). He used suspensions ranging from ordinary agar culture washings to dilutions of 1:100, but he found no marked difference in the size of the galls resulting from any of the preparations. Manifestly, however, even in the 1:100 dilution, the number of bacterial cells must have been in the thousands. The results of this work emphasize the limitations of the dilution method and the indispensability of micrurgical or micromanipulative technique in studies involving small numbers of micro-organisms.

In phytopathology, while the most extensive application of micrurgy has been in connection with the isolation of single cells to obtain pure cultures (*4, 6*), other applications have involved pathological studies of bacterial, virus, and fungus diseases (*1, 5, 7, 8, 10, 12, 13, 14*). Hildebrand (*5*) was the first to demonstrate that a single bacterium is capable of inducing disease in plants.

In animal pathology, although there are reports that as few as one individual tuberculosis germ can produce infection, Webb, Williams, and Barber (*17*) induced tuberculosis in a guinea pig by subcutaneous inoculation with a minimum of 20 tubercle bacilli. They refer to work by Wyssokowicz in which he reported that 8 tubercle bacilli were able to set up an infection in the peritoneal cavity of the guinea pig and that 24 to 30 bacilli were required in the rabbit. They also report that on 4 different occasions 1 thread (3 to 6 bacilli), isolated directly from the blood of a mouse dying from anthrax, caused death when inoculated into healthy mice.

[1] Received for publication November 1, 1941. The work reported here was begun in 1939 at the Rockefeller Institute for Medical Research, Princeton, N. J., during tenure by the writer of a Guggenheim fellowship. The writer was on sabbatical leave from Cornell University in 1939–40, and the work was later completed at Cornell. All the diagrams were prepared by Mrs. D. W. Thomas.

[2] Italic numbers in parentheses refer to Literature Cited, p. 58.

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Thöni and Thaysen (*15*), employing between 10 and 343 tubercle bacilli per inoculation dose, were able to get infection in guinea pigs in but 1 instance, with 71 cells. Although the results of these investigators seem to contradict those of earlier work, it should be mentioned that they used Burri's (*3*) india-ink method, which because of optical difficulties has rarely been used for isolating bacteria, whereas the method of Barber (*2*), employed with some modifications by Webb, Williams, and Barber (*17*), remains even today perhaps the most reliable and widely used technique for isolating single micro-organisms (*6*).

In 1926 Wâmoscher (*16*) conclusively demonstrated for the first time the infectivity of single pneumococcus cells in mice, when 5 out of 21, or 23.8 percent, of the mice that received single cells died from the disease. He obtained a maximum infectivity of 83.3 percent death, with dosages consisting of 11 to 20 bacterial cells per mouse. In making isolations of single cells from blood he used a Peterfi micromanipulator and dark-field condenser. For inoculation the pipette was introduced into a wound in the skin and the tip was broken off underneath the skin surface with a tweezer.

The present paper, an abstract of which has already been published (*8*), reports the results of micrurgical studies with the crown gall organism and attemps to evaluate the significance of wound size and bacterial population in the infection mechanism.

MATERIALS AND METHODS

Bonny Best tomato plants selected for size and uniformity (about 6 inches tall) were used in all experiments.

Several crown gall cultures were used but the chief reliance was placed on the peach strain of N. A. Brown, obtained from Dr. A. C. Braun of the Rockefeller Institute. Highly motile young cultures grown in nutrient media for 9 to 15 hours were ordinarily employed.

The micrurgical apparatus consisted of a double Chambers micromanipulator and accessories (*6*) in modified arrangement for isolation of the bacteria under one microscope and their immediate transfer to the infection court of a plant under a second microscope. Inoculation was accomplished either by a simple shift of the micromanipulator from one microscope to the other or by removal of the pipette containing the inoculum from the holder and manipulating it in the hand during inoculation.

Single-cell isolation was accomplished with the method described by the writer (*6*). By this method one or any desired number of young motile cells could be isolated in preparation for transfer to infection courts.

The smallest wounds tested as infection courts were made into individual living cells in the intact tomato plant with a pipette having a tip diameter of about 3 μ (figure 1). The basal cells in the trichomes or large hairs and the largest epidermal cells adjoining the basal cells of the trichomes on the petiole or stem surface of the tomato were chiefly employed. India ink and olive oil also were injected into living plant cells to check the value of the technique as a means for introducing materials.

The next larger size of wound tested as an infection court was produced by gently stroking the tomato stems once with the side of a polished dissecting needle previously dipped into a bacterial sus-

pension (figure 3, *A*). By this means the trichomes as well as the smaller glandular hairs in the rubbed strip were crushed and small wounds produced both on the hairs themselves and where they joined the epidermis underneath. This operation was performed under a binocular dissecting microscope. The injuries produced appeared to be limited to one or a small number of cells at the base of the hairs. Stems stroked but not inoculated later showed superficial light-colored scars where the hairs had been removed, indicating the very limited character of the injuries.

This method of inoculation was chosen only after nine different ways of producing tiny wounds in individual plant cells or in very small numbers of plant cells on the stem surface had been tried. These

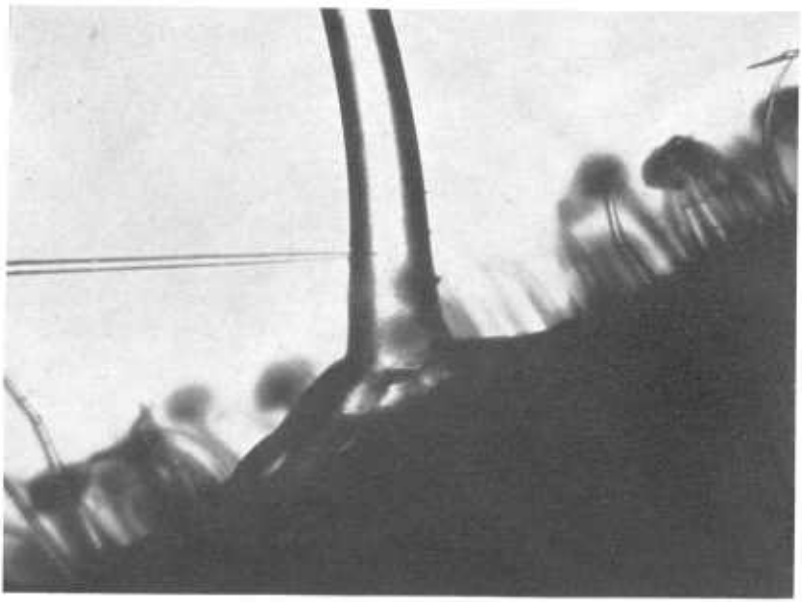

FIGURE 1.—A microinjection pipette inserted into the basal trichome cell on a tomato stem. ×50.

methods of wounding, tested on material under the microscope, were as follows: (1) pinching off the tips of trichome hairs approximately 2 cells away from their basal stem attachment with tweezers that had previously been dipped in a bacterial suspension; (2) pinching off trichomes back to 1 cell away from the stem attachment; (3) removal of trichome hairs with a tiny razor so as not to injure the neighboring basal cells; (4) pulling off trichome hairs with square-tipped tweezers, which often resulted in injury to 1 or more of the neighboring cells; (5) gently stroking the tomato stem with the side of a polished dissecting needle; (6) more vigorous stroking of the needle on the stem surface so as to cause collapse of hairs and injury to epidermal cells underneath; (7) making very shallow wounds by puncturing the epidermis with a small dissecting needle to the depth of 2 to 4 cells; (8) using a similar needle for puncturing the epidermis to a depth of 5 to 8 cells; and (9) using a similar needle for puncturing to a depth of 10 cells or more.

In preliminary tests of methods 1 to 6 the wounding instrument was always dipped in a bacterial suspension so that the amount of inoculum applied was not controlled, although the number of bacteria that got into the microscopic wounds was undoubtedly very small.

Wounding large hairs by methods 1, 2, and 3, always gave negative results, which supports the findings of earlier tests in which individual cells of trichome hairs were injected with bacterial suspensions. Occasional tiny galls resulted in from less than 1 to 10 percent of the trials in wound types 4, 5, and 6, with greatest incidence in 6, where most injury was produced.

Wound types 7, 8, and 9 resulted in gall formation in a relatively higher percentage of cases, apparently because these wounds were larger than the minimal size necessary for infection as represented in wound types 4, 5, and 6.

Deep wounds, to depths of approximately one-fourth, one-half, and completely through the stem, produced under the microscope, were tested as infection courts for different amounts of inoculum. The side of the stems receiving such wounds, as well as those of types 7, 8, and 9, were stroked previously with a smooth needle so as to remove the pubescence, an obstruction which interfered with working on shallow needle-puncture wounds. Following the withdrawal of the needle from both the shallow and deep needle-puncture wounds, sterile juice extract was added from a pipette to provide a protruding meniscus to the wound cavity for facilitating the transfer of inoculum. Immediately after inoculation the plants were placed in a moist chamber for approximately 1 hour to retard the drying of the wound and to allow for penetration by the bacteria.

Sterile juice extract for the purpose indicated and for testing as a growth medium was prepared from the foliage of young, rapidly growing plants. The material was ground in a food chopper, extracted through cloth, centrifuged, filtered through a bacteria-proof filter, and then used immediately or stored in a refrigerator until used.

EXPERIMENTAL RESULTS

GROWTH MEDIA

Juice Extract From Tomato

Sterile juice extract prepared from the stems and leaves of young tomato plants was found to be an excellent medium for culturing single cells of the crown gall organism. Approximately 90 percent of the single cells grew in microculture when transferred to small droplets of the extract of three different preparations of sterile juice. The isolation technique earlier described by the writer (*6*) was used. Three such experiments were conducted which involved the transfer of 9, 11, and 10 active young (10 to 15 hours old) single bacterial cells to as many microdroplets, of which 8, 10, and 10 grew.

The fact that single cells of the crown gall organism grew and multiplied readily in the juice extract from tomato plants indicated that individual bacterial cells should be able to grow also in the plant sap contained in wound cavities of the tomato and possibly even within individual plant cells if such an environment should prove to be congenial and a suitable inoculation technique could be developed.

Wound Sap

Proof that single cells of the crown gall organism had multiplied in the sap in wound cavities were demonstrated by isolation experiments. Five days after deep wounds had been inoculated with single bacterial cells, and before visible evidence of gall formation, wound tissue was dissected from 20 plants, crushed in distilled water in Petri dishes, and poured with nutrient dextrose agar. Seven days later bacterial colonies like those produced by the crown gall organism were noted on 6 plates. In 1 plate there were about 50 colonies, but in the other 5 the colonies were too numerous to count and were estimated to be in the thousands. Representative colonies were inoculated into tomato plants with positive results, leaving no doubt as to the identity of the organism.

As an outgrowth of these preliminary experiments the author set out to discover, if possible, (1) the smallest size of wound that could be used for infecting tomato plants, (2) the smallest number of crown gall bacteria that would produce infection, and (3) the relative influence of number of bacteria in the inoculum and size of wound on the ultimate size of the galls.

SMALLEST SIZE OF WOUND REQUIRED FOR INFECTION

Microinjection into Individual Plant Cells

One hundred and fifty individual plant cells (120 trichome hair cells and 30 epidermal cells) were inoculated by injection with a bacterial suspension (table 1). The micropipette used for inoculation was introduced into the plant cells under the control of a micromanipulator (fig. 1). When the cell wall was perforated with the pipette tip a hemispherical droplet of protoplasm gushed out and then quickly returned into the cell. At the precise moment that the latter process

Table 1.—*Summary of infection experiments involving various types of wounds and amounts of inoculum*

Kind of wound	Tool used	Bacteria used as inoculum	Experiments	Plant cells or stems inoculated	Crown galls induced			Range in radial extension
					Number	Percentage	Range between experiments	
		Number	*Number*	*Number*			*Percent*	*Millimeters*
Microwound (3 μ ±)	Micropipette.	Many	10	150 cells	0	[1] 0		
Microwounds (injury to 1 or more epidermal cells).	Polished needle.	Many	10	500 stems	Many	[2] 5.0		1–2
Shallow wounds (2–4 cells, 5–8 cells, and 10–12 cells deep) grouped [3]	do	1	10	102 stems	10	10.0	0–25	1–7
		2	9	80 stems	8	10.0		
		5	9	72 stems	10	14.0	6–30	
		10	9	90 stems	18	20.0		
		50–100	5	43 stems	9	21.0	10–36	
Deep wounds (one-fourth through, one-half through, and entirely through stem) grouped [3]	do	1	4	40 stems	13	32.0	10–60	10–60
		2	4	40 stems	12	30.0		
		5	4	40 stems	18	45.0	20–90	
		10	4	40 stems	26	65.0		
		50–100	4	40 stems	39	97.5	90–100	

[1] Viable bacteria isolated from only 1 out of 20 tested for survival in inoculated trichome hairs.
[2] Estimated percentage of tiny wounds infected.
[3] The different depths of wounds were grouped together because in failing to control the depth of penetration of the inoculum into the wound by the technique used wound depth lacked significance.

took place increased pressure was put on the pipette contents so as to expel a quantity of the bacterial suspension into the plant cell. Almost immediately after the withdrawal of the pipette what appeared to be a protoplasmic plug filled the wound opening and effectively protected the cell from loss through evaporation. A droplet of india ink was placed on the stem surface adjacent to the inoculated trichome to facilitate its location afterward. Such inoculated cells appeared not to be injured and practically always remained alive, as judged by the fact that protoplasmic streaming continued after the operation. In a few instances particles resembling bacteria could be seen moving in the protoplasmic stream within trichome cells in much the same fashion as do india-ink particles when injected into similar cells.

Not a single instance of gall formation was found in these tests when final observations were made (fig. 2), and 3 weeks after inocula-

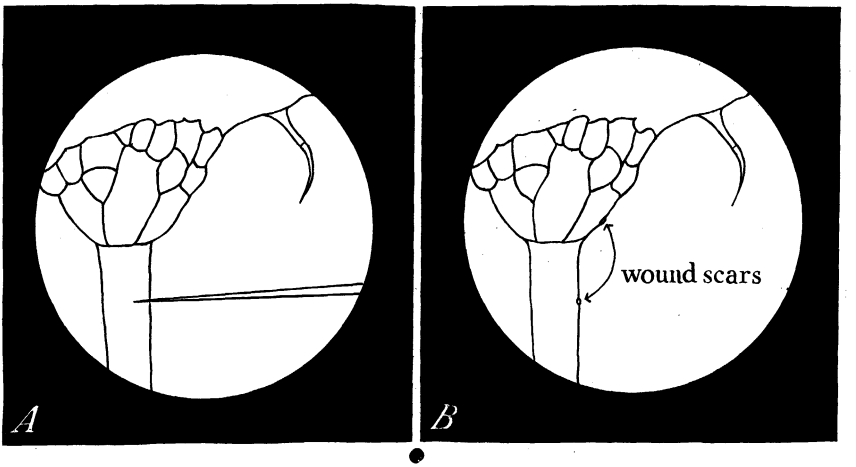

FIGURE 2.—Microinjection of individual trichome cells of tomato with crown gall bacteria: *A*, Many bacterial cells being injected into an individual cell (trichome or epidermal); *B*, at the end of 3 weeks no galls have resulted from the injection and no viable bacteria have been recovered.

tion, many of the trichome cells were still functioning normally. To account for these results several explanations are possible: (1) Wounds approximately 3μ in size may be too small to permit infection if it be assumed that a certain minimum quantity of wound hormone is essential for gall formation; (2) the intracellular environment may not be a favorable medium for these bacteria; (3) the injected bacteria that survived may have been too few or the period of survival too short for stimulating gall formation; or multiplication of bacterial cells, which apparently did not occur, may be essential for gall formation.

ISOLATIONS FROM INOCULATED TRICHOMES

Three weeks after inoculation, virulent crown gall bacteria were recovered from injected trichomes in but 1 case of 20 cultured. The isolations were attempted from 7, 6, and 7 living trichomes from 3 separate inoculation experiments and the crown gall organism was cultured from but 1 trichome in the first series. The hairs were removed by means of a tiny razor soldered to a needle and crushed in a droplet of broth on the inside of a test tube before washing into

a small quantity of sterile broth at the bottom. The interval of 3 weeks was thought to be sufficient for eliminating chance organisms left outside the wound opening in the inoculation operation. However, the single positive case may be the exception to the rule. Since bacteria were isolated in only 1 instance in 20 attempts it would seem that the intracellular environment was not a favorable medium for survival.

Tiny Galls Produced at Tiny Stem Wounds

The smallest wounds in which infection occurred were produced by gently stroking the stems and petioles of tomato plants with a smooth polished needle previously moistened in a bacterial suspension of the crown-gall organism (fig. 3).

Within 5 days after inoculation tiny galls began to appear in close proximity to collapsed hairs and apparently arose from wounds involving one to several epidermal cells. The galls ranged from microscopic size to about 1 mm. in radial extension. Maximum size was reached within about 3 weeks, and forcing the plants by fertilization and planting in deep soil failed to materially increase the size of the galls even after 3 months.

It was estimated that less than 5 percent of the plant cells injured in this experiment became infected. The extent of the injuries depended on the pressure used in stroking and the number of times the needle was passed over a given point. The rubbing operation simultaneously applied a film of bacterial suspension and caused the extrusion of wound sap because of the pressure applied. As soon as

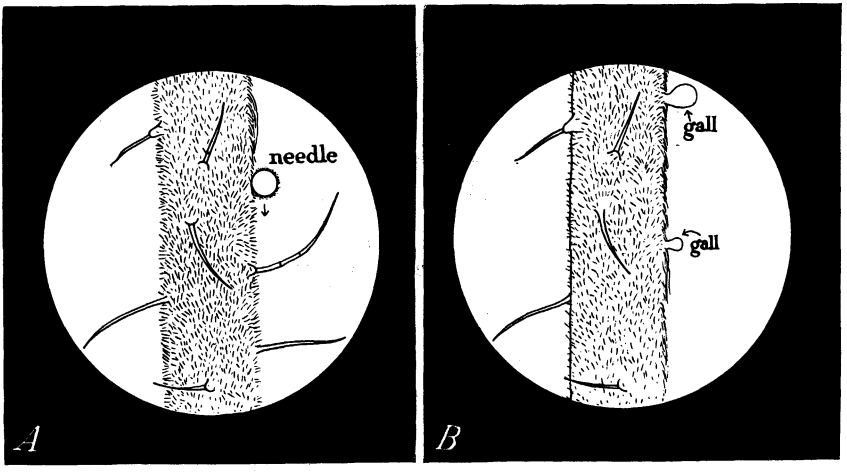

Figure 3.—Rubbing technique of inoculation: *A*, A needle dipped in bacterial suspension was stroked gently over the stem surface, resulting in minute wounds involving one to several plant cells; *B*, occasional tiny galls formed in proximity to the collapsed hair cells, such galls reaching a radial extension of about 1 mm.

the pressure was removed some of the sap and bacterial mixture returned into the wound.

From the results obtained it appeared that the small size of the galls may have been due to the minuteness of the food supply in the wounds and the relatively few bacterial cells that could gain entry and survive therein.

Bacterial Population in Tiny Galls

Estimates of the number of bacterial cells were obtained by the standard dilution agar plate technique. Individual galls of several ages and sizes were used with or without a washing treatment to remove the bulk of chance organisms present on the outside. Excess water was blotted off the galls by means of sterilized paper toweling and entire galls were removed, cutting tangentially to the stem surface, with a razor. Each gall was ground fine in a sterile mortar in a small quantity of water before dilution plates were poured. It was soon found that dilutions were ordinarily unnecessary because of the small yield of bacteria. After an incubation period of about 4

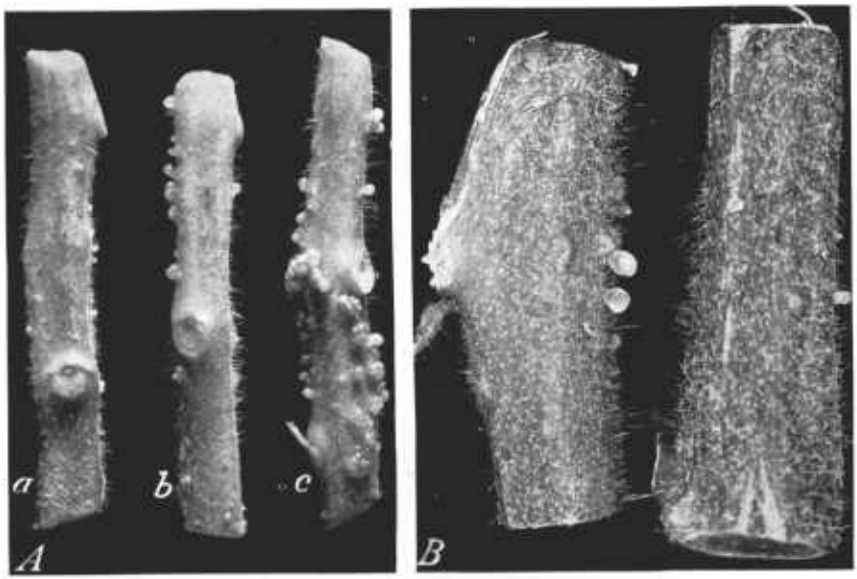

Figure 4.—*A*, Tiny galls induced by rubbing tomato stems with a polished needle gently (*a*) and progressively harder (*b*) and (*c*). The increase in size of wounds was correlated with an increase in the size of the galls induced. ×0.9. *B*, Enlarged view of two stem pieces with similar tiny galls. ×3.

days colonies resembling the crown gall organism were counted, and representative samples were used for pathogenicity tests to confirm the diagnosis.

On the whole, the number of bacteria isolated from tiny galls was very small, averaging less than 100. In 1 series of experiments the 54 galls cultured gave a total of 1,691 colonies, or an average of 31 colonies per gall, while the yield per gall ranged from 0 to 550 colonies. Only 4 galls yielded over 100 colonies. In another study 90 tiny galls yielded a total of 10,582 colonies or an average of 118 colonies per gall. Of these 1 gall yielded 5,520 or over half of the total; a second gall gave 1,223 colonies; 7 galls gave between 100 and 1,000 colonies; 34 galls less than 10 colonies; and 29 galls gave none. Failure to obtain any bacteria in about one-third of the galls suggests their elimination either through the technique employed or by death due to an unfavorable environment. It is possible that some of the galls negative for bacteria in the isolation tests are representative of what

is obtained when crown gall bacteria get inside living tomato cells or, as in the case of sunflower (*18*), when bacteria-free secondary galls occur at a distance from the point of inoculation. While the inability of crown gall bacteria to survive within injected cells, already mentioned, supports this conclusion, more work is needed on this point.

Location of Bacteria in the Galls

The approximate location of the crown gall bacteria in the tiny galls was studied by pouring plates of galls divided into two or three parts under the microscope by means of a microtool fashioned from a safety razor blade. Three experiments were conducted.

In the first experiment isolations were attempted from the tip one-third part of 7 tiny galls, and the results were positive in 2 cases. At the same time isolations made from 11 complete galls gave positive results in all cases.

The second experiment involved isolations from 10 tiny galls divided into 2 approximately equal parts. All galls yielded bacteria—the tip half in 9 cases and the basal half in 4 cases.

In the third experiment eight galls were divided into three approximately equal parts. Of the eight galls seven yielded pathogenic bacteria. Bacteria were obtained from the tip, middle, and basal regions respectively of three, five, and four of these tiny galls.

The results of the foregoing tests show that bacteria may be found in various parts of the tiny galls and are not limited to one location.

Smallest Number of Bacteria Required to Induce Gall Formation

Shallow Needle-Puncture Wounds

When one or more bacterial cells were used as inoculum and shallow stem wounds (about 0.1 mm. in diameter) of three depths (2 to 4 cells deep, 5 to 8 cells deep, and 10 to 12 cells deep, approximately) were employed as infection courts, infection occurred in a maximum of 21 percent of the trials (table 1).

Single bacteria induced gall formation in about 10 percent of the plants inoculated, as compared with approximately 15 percent for 2 to 10 bacterial cells and 21 percent for 50 to 100 bacterial cells (fig. 5).

The galls induced by single cell inoculation began to appear in about 1 week after inoculation and reached maximum size in 3 weeks. They were usually small, ranging from less than 1 to about 4 mm. in radial extension at the end of 3 months (fig. 6*A*). There was a tendency for the galls to become larger as the depth of the wound increased.

The galls induced by inoculation with 2 to 10 bacterial cells finally reached between 1 and 5 mm. in radial extension. Again the larger galls were associated with the deeper wounds.

When between 50 and 100 bacterial cells were introduced into shallow wounds the majority of the resulting galls reached between 1 and 7 mm. in radial extension and were larger for the greater wound depths.

From these results it is apparent that infection was somewhat higher and the galls were larger when more than one bacterial cell was used as inoculum and deeper wounds were inoculated. This slightly higher percentage of infection where more than one bacterial cell was used, while significant, is believed to be the result of chance. The probability of a single bacterium, as contrasted with two or more cells, reaching

the proper location in the wound for setting up an infection was undoubtedly less; therefore the percentage of infections would be expected to be smaller for the single cell. Moreover, one or more cells of an inoculum consisting of several cells would have a better chance of reaching a greater depth, as well as a better position for multiplication, than would a single cell. Consequently a correlation between size of gall and depth of wound was expected. Basic to the above interpretation is the well-known tendency of the crown gall

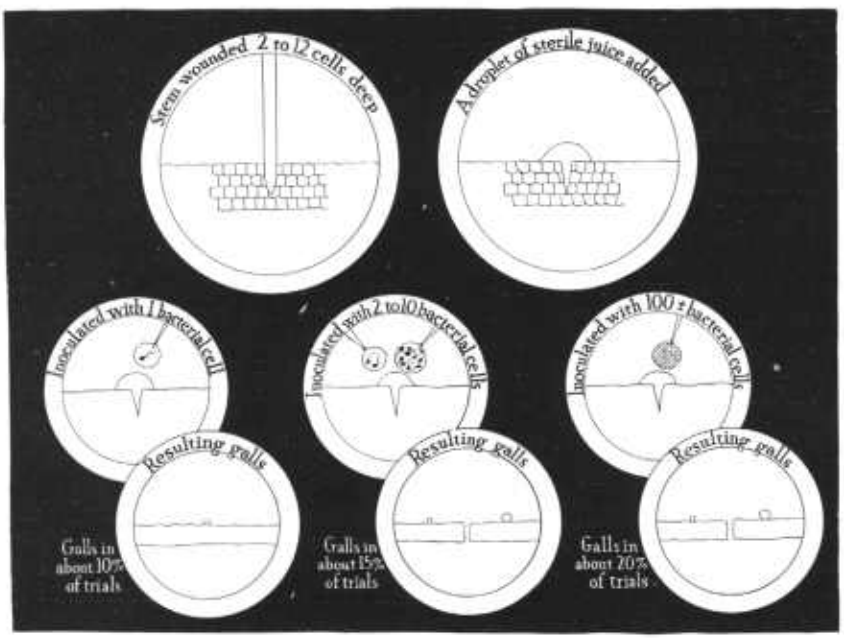

FIGURE 5.—Inoculation of shallow needle-puncture wounds with one or more crown gall bacteria, showing production of small galls.

organism to localize in the tissue and to stimulate only a limited number of the surrounding cells to activity.

DEEP NEEDLE-PUNCTURE WOUNDS

Deep wounds made by using the same size needle as before proved much more efficient as infection courts than shallow wounds (table 1). Three types of deep wounds (needle punctures one-fourth, one-half, and completely through the stems) were tested, one or more bacterial cells being used for inoculum. Single bacteria produced infection in from 10 to 60 percent of the trials (fig. 7); 2 to 10 bacteria produced galls in from 20 to 90 percent of the trials, and 50 to 100 bacteria produced galls in practically all the trials. In every case the galls were larger in radial extension than the thickness of the stems. There was a definite correlation between depth of wound and size of gall since the largest galls were always associated with needle punctures all the way through the stems (fig. 6, *B*). However, no apparent correlation was found between amount of inoculum and

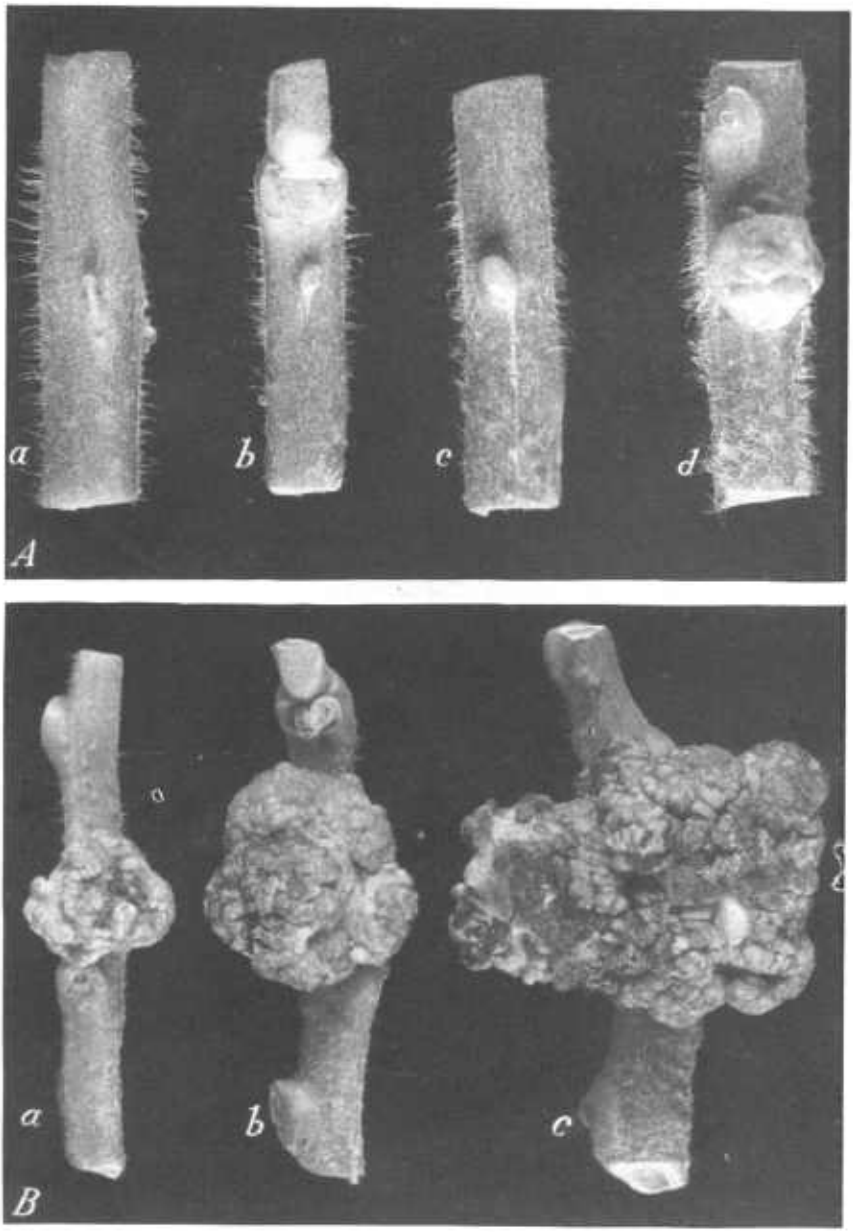

FIGURE 6.—Crown galls induced by inoculation with single bacteria: *A*, Maximum size attained in 3 months by crown galls induced by single-cell inoculation into wounds 2 to 4 cells deep (*b*); 5 to 8 cells deep (*c*); and 10 to 12 cells deep (*d*), as compared with a tiny gall (*a*) induced by inoculation of a very small epidermal wound resulting from the removal of a single trichome hair. × 1.4. *B*, Maximum size attained at the end of 3 months by galls induced by the introduction of single bacteria into wounds one-fourth (*a*), one-half (*b*), and completely through (*c*) the tomato stem. × 0.9.

size of gall. The reason for the single cell being less infective than larger numbers of cells on a percentage basis was undoubtedly due to the fact that there was less chance of one cell finding a favorable place for multiplication in the wound cavity. However, if a bacterium once found congenial surroundings, as in a deep wound, it proved actually equal in potentialities to larger numbers of cells so far as gall size, the end result, was concerned.

It has already been shown that one bacterium can quickly multiply into large numbers in sap extract and that the sap in the wound cavity functions in the same way as the food supply in microculture or in a test tube. The principal limiting factor governing gall size in these experiments was food supply for the increase of the bacterial population and not the initial amount of inoculum. Therefore, it was

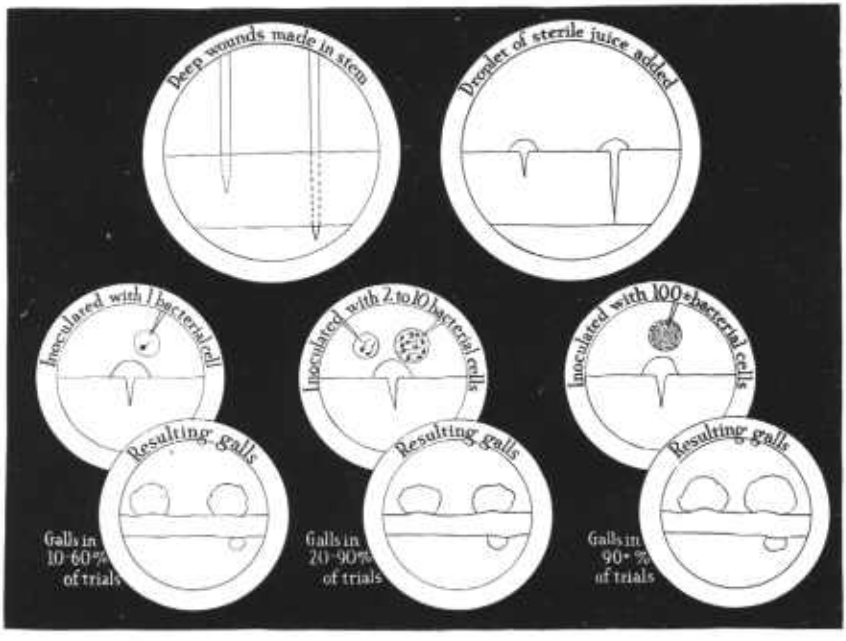

FIGURE 7.—Inoculation of deep needle-puncture wounds with one or more crown gall bacteria showing production of large galls.

concluded that the kind of wound was a more important factor in producing infection than the initial number of bacteria.

BACTERIAL POPULATION AND SIZE OF GALL

Isolations made from galls of various sizes taken at random from the several experiments in which shallow and deep needle-puncture wounds were employed indicated that bacterial population is roughly proportional to gall size. Small galls ranging from 1 to 3 mm. in radial extension yielded an average of between 50 and 100 colonies per gall in isolation trials. Always the entire gall was crushed in water in a Petri dish and allowed to stand for an hour before the agar was added. Galls from 5 to 7 mm. yielded between 100 and 50,000 colonies.

Galls 7 mm. and upwards ordinarily gave counts averaging 100,000 or more. One series of 7 galls measuring from 10 to 25 mm. in diameter yielded in round numbers 1,106,500; 400,000; 1,143,000; 1,070,000; 510,000; 11,000,000, and 20,000,000 bacteria based on the plate counts.

DISCUSSION

The present investigation, which involved the use of micrurgical technique, demonstrated the fact that a single cell of the crown gall organism when introduced into a wound is capable of producing infection in tomato. Apparently such potentiality of individual bacteria had been previously demonstrated in but one instance in animals (*16*) and one in plants (*5*).

The greatest difficulty encountered in this study was not that of developing precision technique for isolating and transferring known numbers of bacteria to the wounds, but rather that of introducing the isolated bacterial cells into the proper position in the needle-puncture wounds for infection to take place. Filling the wounds with sterile juice extract and then planting the inoculum on the protruding meniscus was found to be inadequate for the proper distribution of inocula in the shallow wounds, but proved much more efficient for the deep wounds. The interpretation given to this seeming discrepancy was that of the role of chance in distributing the bacteria in the wounds.

The complete failure of microscopic pipette wounds to become infection courts probably cannot be charged to lack of perfection of the microinjection technique. This conclusion is supported by the evidence from isolation experiments in which it was found that virulent cultures failed to survive the intracellular environment. Thus far no one has given convincing evidence that living crown gall bacteria are ever present in living plant cells. One of the most recent studies was that of Banfield (*1*) who reported negative evidence on this point. The final answer to this question will require further work.

SUMMARY

Juice extract from tomato plants was found to be an excellent medium for culturing the crown gall organism. Single bacteria grew readily in the juice extract in microculture.

The sap in the wound cavity liberated from the cells that were injured in the wounding operation also supported growth of the crown gall organism. Isolations made from wounds about 5 days after inoculation and before symptoms appeared, showed that the original single cells had multiplied into thousands of individuals in the wound sap.

The crown gall organism, when injected into the living cells of tomato stems, failed to induce gall formation and ordinarily failed to survive inside the cells, indicating that the living cell interior is an unfavorable medium for these bacteria.

Single bacterial cells induced gall formation when introduced into needle-puncture wounds of various sizes. The lower percentage of infections resulting from single-cell inoculation as contrasted with that from inocula consisting of larger numbers, was attributed to the role played by chance in distribution which favored the larger num-

bers reaching the proper position for multiplication and cell stimulation in the wounds.

Tiny wounds in the tomato stem, involving one or more epidermal cells, approximated the minimum size for infection by the crown gall organism. Only a small percentage of such wounds became infected when the inoculum was applied by gently rubbing the stem surface with a polished needle moistened with a bacterial suspension.

Shallow stem wounds (from about 2 to 12 cells deep) were less efficient as infection courts than deep wounds (from one-fourth to completely through the stem) when the inoculum was identical. This result was attributed to the role played by chance in the distribution of the bacteria and to the larger amount of wound sap which favored the larger wounds as infection courts.

Ordinarily the ultimate size of the gall was correlated with the depth of the wound but was independent of the size of the initial inoculum. The largest galls observed resulted from inoculating deep stem wounds regardless of whether the inoculum was a single bacterium or large numbers of bacteria.

The relation (1) between tiny wounds, tiny galls, and few bacteria, (2) between shallow wounds, small galls and more bacteria, and (3) between deep wounds, large galls, and many bacteria was verified by isolation experiments.

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